Assessing global regionalized impacts of eutrophication on freshwater fish biodiversity

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Chapter 4

Effects of nitrogen emissions on fish species richness across the world's freshwater ecoregions
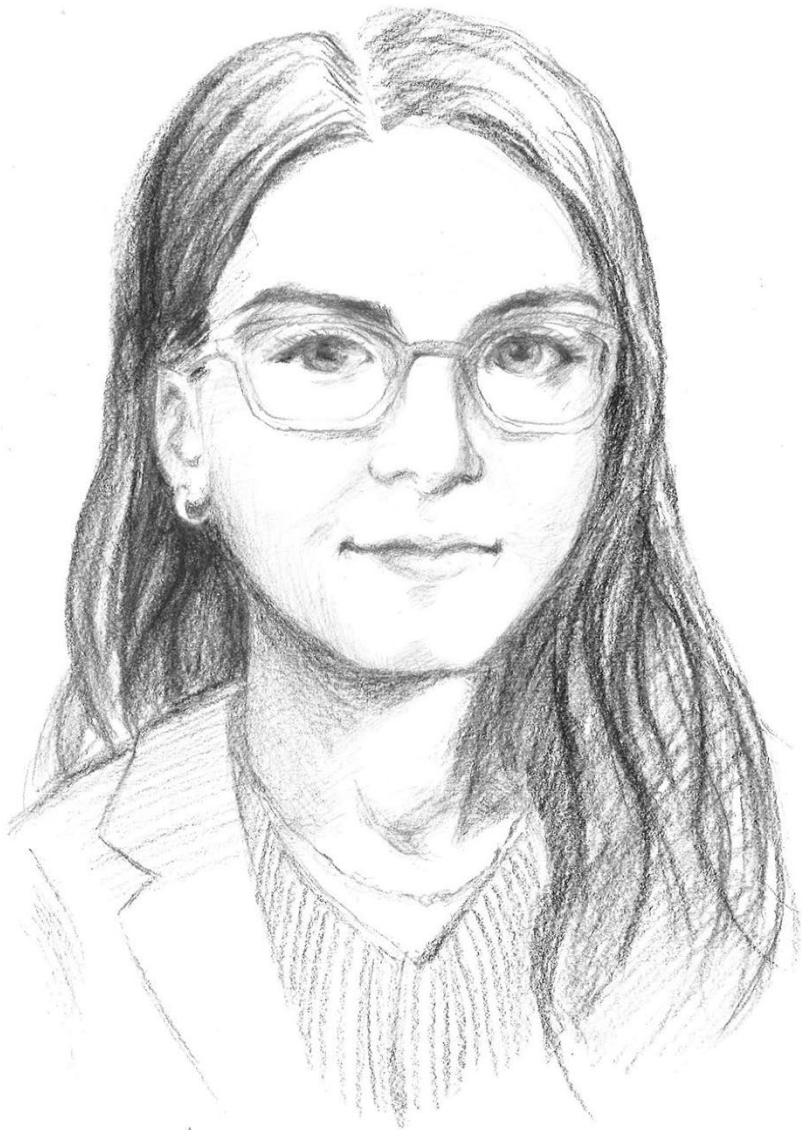
Laura Scherer

Abstract

The increasing application of synthetic fertilizer has tripled nitrogen (N) inputs over the twentieth century. N enrichment deteriorates the water quality and threatens aquatic species such as fish through eutrophication and toxicity. However, N impacts on freshwater ecosystems are typically neglected in Life Cycle Assessment (LCA). Due to the variety of environmental conditions and species compositions, the species' response to N emissions differs among ecoregions, requiring a regionalized effect assessment. Our study tackled this issue by establishing regionalized species sensitivity distributions (SSDs) of freshwater fish against N concentrations for 367 ecoregions and 48 combinations of realms and major habitat types globally. Subsequently, effect factors (EFs) were derived for LCA to assess the N effects on fish species richness at a 0.5×0.5-degree resolution. Results show good fits of the SSDs for all the ecoregions with sufficient data and similar patterns for average and marginal EFs. The SSDs highlight strong effects on species richness due to high N concentrations in the tropical zone and the vulnerability of cold regions. Our study revealed the regional differences in sensitivities of freshwater ecosystems against N content in great spatial detail and can be used to assess more precisely and comprehensively nutrient-induced impacts in LCA.

4.1 Introduction

Global food production has tripled in the past five decades to meet the demand of growing populations (FAOSTAT, 2008; Grübler et al., 1995). This has been achieved using large amounts of synthetic fertilizer for cultivating crops (Smil, 1999), importing excessive nitrogen (N) into local nutrient cycles (Bouwman et al., 2009; Galloway et al., 2004; Mogollón et al., 2018b). During the latter half of the 20th century, the global use of nitrogen fertilizers grew seven-fold (Smil, 1997), leading to a tripling of nitrogen inputs into freshwater systems (Zhang et al., 2021). This input reached approximately 120 Tg N yr^{-1} in 2000. It represents a combination of fertilizer, manure, biological N2 fixation, and nitrogen deposition. Future scenarios posit that N inputs will continue to rise due to population growth and the increasing proportions of proteins in human diets

(Beusen et al., 2022; Mogollón et al., 2018b; Smil, 2002).

The excessive release of nitrogen into the environment is adversely affecting ecosystems (Jenny et al., 2016; Schindler, Vallentyne, 2008; Vonlanthen et al., 2012). For instance, N enrichment can induce eutrophication in water bodies and toxicity to species. Eutrophication can cause hypoxia, the severity of which determines the survival of aerobic organisms in the water. Moreover, nitrate (NO_3^-) in drinking water is not only harmful to human health (Bryan and van Grinsven, 2013), but ammonia (NH_4), NO_3^-, and nitrite (NO_2^-) also perturb the pH and are toxic to fish (Russo and Thurston, 1977; Thurston et al., 1981; Westin, 1974). Many fish species are top predators, and their survival, diversity, and health are good indicators of the functioning of aquatic ecosystems (Villéger et al., 2017; Whitfield and Elliott, 2002).

Life cycle assessment (LCA) can be used to characterize the impact of eutrophication on biodiversity (Payen et al., 2019). LCA is a tool for assessing the environmental impacts of products across their life cycles and can help analyze the trade-offs between economic activities and the environment (Hunkeler, 2014; Muralikrishna and Manickam, 2017). Within the life cycle impact assessment (LCIA) phase of an LCA, characterization factors (CFs) express the relative magnitude of a certain environmental impact per unit of the characterized activity (Rosenbaum et al., 2018). As an endpoint-level component of CFs, effect factors (EFs) describe the sensitivity of the species community to environmental pressure (Potting and Finnveden, 2015). Such EFs can be used to assess N effects on freshwater biodiversity (Payen et al., 2019). Cosme and Hauschild (2017) estimated the effect of marine eutrophication-induced hypoxia on species. They used dissolved oxygen (DO) as an intermediate factor (exposure factor) connecting N with effects on species. Azevedo et al. (2013) investigated the patterns of biodiversity along phosphorus (P) concentration gradients in lakes and streams based on limited data from peer-reviewed papers. LC-IMPACT (Azevedo et al., 2020), ReCiPe2016 (Huijbregts et al., 2017), and Jwaideh et al. (2022) applied EFs derived from the P-species sensitivity distributions of Azevedo et al. (2013) that directly link to the fate of the nutrient (i.e., without exposure as an intermediate factor). However,

Cosme and Hauschild (2017) focused on marine ecosystems and only considered the hypoxia induced by N yet ignored the effect of N toxicity on species, while Azevedo et al. (2013) and the following phosphorous EF studies have not accounted for N. Furthermore, both of these studies were conducted at a very coarse scale of distinguishing the effects only for four or five biogeographical regions globally. In various cases, N contributes strongly to freshwater eutrophication. Globally, 26% of the area with undesirable periphyton growth is limited by N compared with 74% for P. When considering acceptable and undesirable periphyton growth, even 66% of the area is limited by N compared with 34% for P (McDowell et al., 2020). However, N effects on the ecosystem have not been explored globally (Payen et al., 2019; Zhou et al., 2022b).

Hydro-climatic and morphological conditions specific to catchments/ecoregions dictate patterns of fish distribution (Schipper and Barbarossa, 2022). Therefore, the response of fish assemblages to human stressors is contingent on these environmental conditions (Larentis et al., 2022; Schipper and Barbarossa, 2022). For instance, variation in water temperature may change dissolved oxygen demand for respiratory purposes for organisms (Kramer, 1987). Meanwhile, biotic sensitivity to such hypoxia differs between species. Therefore, N-induced eutrophication has divergent effects on diverse species composition across distinct ecoregions (Clark et al., 2017; Ekau et al., 2010; Larentis et al., 2022). Regionalized EFs of fish biodiversity loss are therefore required to describe the N impact on fish diversity across different ecoregions.

This study aimed to explore the regionalized effects of N on global freshwater ecosystems. Based on 41 years of fish occurrence data (covering 13,920 freshwater fish species) and N concentration simulations from the Integrated Model to Assess the Global Environment – Global Nutrient Model (IMAGE-GNM) (Beusen et al., 2015), we calculated the fish species sensitivity distribution (SSD) over 367 ecoregions and provide EFs of potential N-induced species loss at a half-degree resolution. This study is the first to reveal the statistical relationships between N content and species loss in the global freshwater system.

4.2 Methods

4.2.1 Global nutrient model

Measurement data for nutrients in global rivers and lakes are rare, especially for N. Worldwide total N (TN) sampling stations only occupy less than half of water quality stations (e.g., 4,685 N sampling stations out of over 18,000 river stations from the GLObal RIver CHemistry Database, GLORICH (Hartmann et al., 2019)) and cover few regions (e.g., only 83 countries are covered by N stations in the global water quality database, GEMStat (UNEP GEMS/Water Programme, 2007)).

The use of a global nutrient model can fill the gap of a lack of spatial N information, as it can predict the unknown N concentrations in water bodies without sampling (Beusen et al., 2016; Kroeze et al., 2012; Mayorga et al., 2010). Among recognized global nutrient models, IMAGE-GNM is a spatially explicit, dynamic model with the finest resolution (0.5×0.5 degree) that has been validated with sampling station data (van Vliet et al., 2019). The validation of Zhou et al. (2022a) found a NRMSE of 2.29 and a Pearson correlation coefficient (r) of 0.58 of IMAGE-GNM generated estimates vs. 9,770 records of total nitrogen observed data from 1,199 global river stations. Of these, the North Frigid Zone performed best in terms of NRMSE (0.57) but had a low r (0.14); the North Temperate Zone performed best in terms of r (0.59) but had a high NRMSE (2.35); the Torrid Zone had a relatively low NRMSE (1.71) but the lowest r (0.05); and the South Temperate Zone generated a relatively high r (0.46) but the second highest NRMSE (1.91) (The scale of these regions can be found in "Geographical zone" (2009)). In this study, we employed IMAGE-GNM to provide global N concentration estimates from 1970 to 2010 (Beusen et al., 2016, 2015; Bouwman et al., 2009), as these are the most recent years that are accessible from IMAGE-GNM. A detailed model description of IMAGE-GNM can be found in Beusen et al. (2015).

4.2.2 Freshwater fish species inventory

We compiled point occurrence data for freshwater fish species following the same

methodology as in Barbarossa et al. (2021, 2020). First, we retrieved occurrence data from the Global Biodiversity Information Facility (GBIF) (GBIF.org, 2019), FishNet network (Fishnet2, 2019), SpeciesLink (splink, 2019), Portal da Biodiversidade (ICMBio, 2019), and the Atlas of Living Australia (ALA) (PLAN, 2008). An overview of these source data can be found in Table S1 of Barbarossa et al. (2020). Second, we coupled the fish occurrence data to freshwater fish species names and associated synonyms, which were derived from Fishbase (we used the R package "rfishbase 4.0.0" (Boettiger et al., 2012) and set FISHBASE_VERSION as "19.04") and Tedesco et al. (2017). Based on these datasets, we harmonized freshwater fish species names and excluded the occurrence records without year and geographic information. This step selected 13,774 unique freshwater fish species with scientific names and 825 species with synonyms. Third, we merged these occurrence records by assigning the scientific names for those with synonyms and removing the duplicates. In total, we obtained 13,920 freshwater fish species and 5,427,740 occurrence records from 1970 to 2010. Details can be seen in Supporting Information Figure S4.2.

4.2.3 Species sensitivity distributions

EFs (PDF·m^3·kg^{-1}, described in section 4.2.4) are used within life cycle impact assessment (Rosenbaum et al., 2007) and typically expressed as the potentially disappeared fraction (PDF) of species, i.e., the relative species richness as a fraction of the total, per unit of an increase in stressor. EFs reflect both the sensitivity of the species (PDF between 0 and 1) and the size of the system being affected (here: volume). EFs can be derived from species sensitivity distributions (SSDs), which represent the continuous relationship between PDF and a stressor. In this case, PDF (dimensionless, Eq. 4.1) depends on the loss of species richness under the influence of N concentration levels (mg·L^{-1}) compared to the maximum species richness that can be observed within ecological units. N can stress ecosystems through both eutrophication and toxicity. As an ecological unit, we used freshwater ecoregions, which are deemed a characteristic, geographically distinct combination of natural communities (Abell et al., 2008). Thus

we set ecoregions as the smallest ecological units to model EF (Supporting Information Figure S4.1, 426 freshwater ecoregions over the globe, data from Freshwater Ecoregions of the World (FEOW)). We also employed a coarser biogeographical classification by combining the realms and the major habitat types as a supplement to some ecoregions that lack occurrence records (49 realm-major habitat types globally, of which 48 have sufficient data to support regressions). The ecoregions and realm-major habitat types were rasterized before the derivation of SSDs. When the number of pairs of PDF-N concentration data was three or fewer, we deemed it insufficient for fitting SSD curves.

$$PDF = 1 - \frac{SR_{i,j}}{SR_{i,max}} \qquad (4.1)$$

where $SR_{i,j}$ is the species richness of ecoregion i at the N-concentration level j and $SR_{i,max}$ is the maximum species richness in ecoregion i.

We extracted the species richness by counting the number of fish species that can survive at a given N concentration level for each ecoregion/ realm-major habitat type. We assume that the fish species are tolerant to the prevailing N-induced hypoxia/toxicity up to the N concentration at which they are observed within an ecoregion, whereas fish species richness gradually decreases with increasing N concentration by exceeding the species' tolerance levels. This follows a similar approach to Gade et al.[61] for terrestrial acidification. To keep consistency with current LCA practices, the lower tolerance threshold was not considered. The lower tolerance threshold ensures organisms do not starve from a lack of nutrients. This threshold is beyond the scope of this study and may contain a functionally distinct ecosystem from the reference system (Payen et al., 2019). Therefore, we only consider the upper threshold of the stressor.

By matching the species occurrences with N concentration of the same year and location (by pixels of 0.5×0.5 degree), we derived the N tolerance thresholds for each species within each ecoregion/ realm-major habitat type.

We can predict SSD curves with a logistic function (Eq. 4.2) to fit the data pairs of PDF (calculated in Eq. 4.1) and N concentration. This function is widely used in LCIA method development (Azevedo et al., 2013; Gade et al., 2021; Roy et al., 2014; Scherer et al., 2022).

$$PDF = \frac{1}{1 + \exp\dfrac{(a - log_{10}C_N)}{b}} \tag{4.2}$$

where a and b are empirical coefficients, a indicates the N concentration at which 50% of the species have disappeared, and b can be interpreted as the slope of the SSD. C_N (mg·L^{-1}) is the N concentration.

We evaluated the performance of the regression using the pseudo-R^2 value and the normalized root mean square error (NRMSE). Normal R^2 values for linear regressions have been shown to be inappropriate for non-linear fits (Spiess and Neumeyer, 2010). We, therefore, selected Cox-Snell pseudo-R^2, one of the most commonly used R^2 for non-linear regressions (Smith and McKenna, 2013). This index compares the likelihood ratio of the fitting function model to a null model that contains only the intercept. NRMSE discloses the magnitude of the errors normalized by the average value through division. We regarded as a good fit a Cox-Snell pseudo-R^2 > 0.5 and NRMSE < 1, following Scherer et al. (2022) and nutrient model research (Nakhaei et al., 2021; Zhou et al., 2022a).

4.2.4 Effect factors

Two main approaches can be used to calculate EFs: marginal and average.

In this study, we employed both approaches to show different perspectives of the N effects on fish biodiversity. The marginal approach ($EF_{marginal}$, PDF·m^3·kg^{-1}, Eq. 4.3) denotes the instantaneous change of effect due to the current stressor and is calculated as the derivative of the SSD (i.e., Eq. 4.2) at the current state.

$$EF_{marginal} = 1000 \cdot \frac{\mathrm{d}PDF}{\mathrm{d}C_N} = 1000 \cdot PDF^2 \cdot \left(\frac{b \cdot \exp\dfrac{(a - log_{10}C_N)}{b}}{C_N \cdot \ln(10)} \right) \qquad (4.3)$$

where 1000 is the coefficient to convert the unit of the reciprocal of C_N from $L \cdot mg^{-1}$ to $m^3 \cdot kg^{-1}$.

The average approach ($EF_{average}$, $PDF \cdot m^3 \cdot kg^{-1}$, Eq. 4.4) represents the long-term change of effect, comparing the current state with a desired reference state. This could be a state without anthropogenic interference, a political target, or a zero effect, and in this study, we took the year 1900 as the reference year, with the N concentrations taken again from IMAGE-GNM. The average approach has also been used to assess the effect of future states under different greenhouse gas concentration trajectories (de Schryver et al., 2009; Scherer et al., 2022).

$$EF_{average} = 1000 \cdot \frac{PDF_{current} - PDF_{ref}}{C_{N,current} - C_{N,ref}} \qquad (4.4)$$

We calculated globally marginal and average EFs based on the gridded N concentration at the current state (represented by the year 2010) at a resolution of 0.5×0.5 degrees and the SSDs for the corresponding ecoregion/realm-major habitat type. EFs were derived from ecoregion-level SSDs first, and the realm-major habitat type was employed only if the species-stressor information was not sufficient for the ecoregions.

In those regions with zero N concentration, EFs were set to no value because no SSD could be derived. We also regarded N concentration < 0.0001 $mg \cdot L^{-1}$ as zero N concentration due to the uncertainty in measurements and modeling.

4.3 Results

4.3.1 Species sensitivity distributions and potentially disappeared fractions

Among the 426 ecoregions, SSD curves could be derived for 367 ecoregions, and all of them performed well (Cox-Snell pseudo-$R^2 > 0.5$ and NRMSE < 1). Data from 22

ecoregions were insufficient to fit SSD curves (PDF-N concentration data pairs $\leq$ 3), and 37 ecoregions did not have any data. The minimum Cox-Snell pseudo-R^2 was 0.57, found for Lake Tanganyika (NRMSE = 0.36) and the maximum NRMSE equalled 0.55, found for Chuya (Cox-Snell pseudo-R^2 = 0.75). In total, the 367 analyzed ecoregions occupy 95% of the global area. Among them, 357 ecoregions had a Cox-Snell pseudo-R^2 > 0.8 and NRMSE < 0.4 (The maps of Cox-Snell pseudo-R^2 and NRMSE can be found in Supporting Information Figure S4.3 and S4.4). Ecoregion-level SSD curves of 6 of the large ecoregions of different continents are shown in Figure 4.1 as examples.

For those 59 ecoregions without ecoregion-level regression, we provided the realm-major-habitat-type-level SSDs. With these SSDs, we can fill the gaps of 58 of these ecoregions (Cox-Snell pseudo-R^2 > 0.5 and NRMSE < 1). The remaining ecoregion Bermuda, which belongs to Nearctic-Oceanic Islands, had data for neither the ecoregion level nor the realm-major habitat type level. The 58 SSD plots for the realm-major habitat type can be found in Supporting Information 3, and an overview of regression coefficients, criteria, area, etc. of ecoregions and realm-major habitat type are listed in Supporting Information 4 in Zhou et al. (2023).

Figure 4.1 illustrates the PDF of the current state (2010). The regions with zero N concentration and consequently no value for PDF occupy 14% of the global area. These regions are remote and included several lakes and arid zones (e.g., Sahel Desert and Australian deserts). In the regions with non-zero N concentration, about 15% of the area is at severe risk of potential disappearance of the local fish communities (PDF > 0.8). Among these, the high PDF of ecoregions might mainly be caused by high N emissions to freshwater over the years due to a large increase in population density (e.g., Lower Yangtze River in China and Southern Deccan Plateau in India), while the high PDF of a few regions in polar freshwater systems might result from the uncertainty raised by a lack of observational data, e.g., fish biodiversity of Lena and Taimyr showed high sensitivities to N content since all the occurrence was recorded at low N concentrations. The changes in PDF and N concentration can be found in Figure S4.5 and Figure S4.6 in Supporting Information.
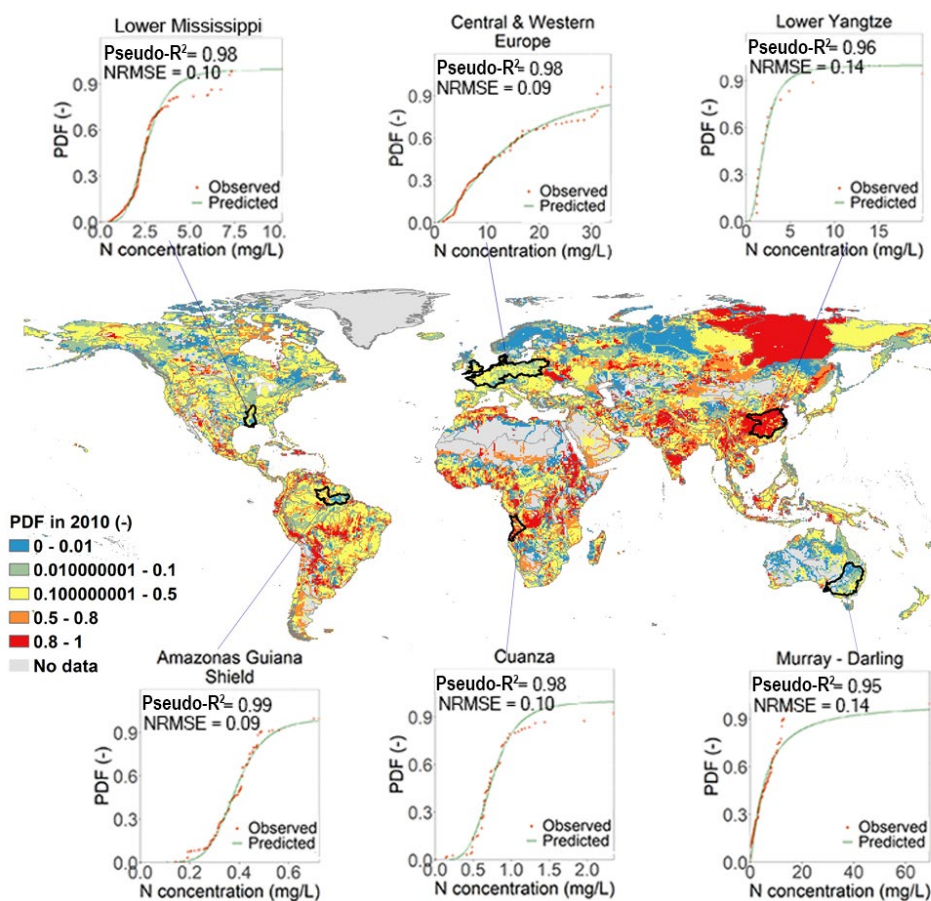
Figure 4.1 Potentially disappeared fraction (PDF) of the current fish species diversity (2010) at a 0.5×0.5° resolution and species sensitivity distributions (SSDs, following Eq. 4.2) for examples of large ecoregions in 6 continents. Note that the right limit is contained, while the left limit is not contained in the segment; e.g., 0 – 0.01 means 0< PDF ≤0.01.
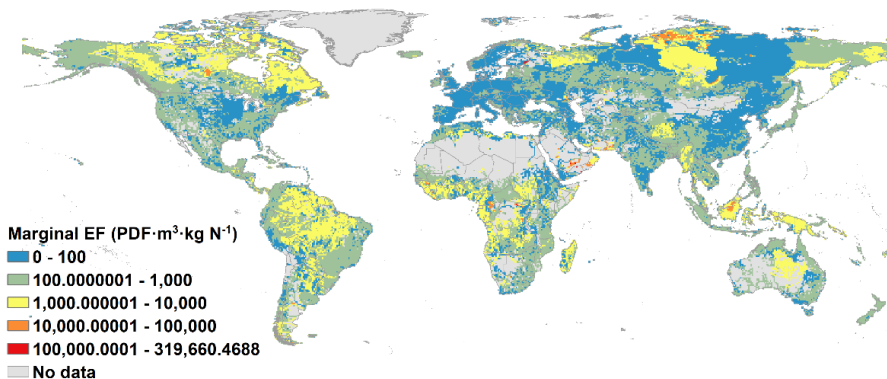
4.3.2 Effect factors

Marginal EFs and average EFs showed similar spatial patterns (Figure 4.2 and Figure 4.3). High values for marginal and average EFs (>100,000 PDF·m^3·kg^{-1}) occurred in less than 1% of the non-zero N concentration area and were distributed in, e.g., Taimyr, Arabian Interior, Baluchistan, Borneo Highlands, and Sangha. Despite the similarity,

marginal EFs include slightly more areas with high values ($>100{,}000$ PDF·m^3·kg^{-1}) and low values (≤ 100 PDF·m^3·kg^{-1}), i.e., 33% had low values and 1.2% high values, while the average EF had 29% and 0.6% of low and high values, respectively.

The reason for more areas with high values for marginal EFs is that the current state is at a stage of a rapid increase of PDF, while the average EFs smoothen the change of effect by taking the difference between the current and desired state. In particular, the marginal EF showed an accelerated species sensitivity at PDF ≤ 0.5 (occupying 70% of the area).

The higher percentage of low values in marginal EFs was caused by situations where PDF approaches 0 and 1, as, at these stages, little change of species richness can happen under the perturbation of N content in water. When the current PDF is close to 1, average EFs can show higher values than marginal EFs, as long as N concentrations varied from 1900 to 2010 in those regions. For instance, an average EF > 100 PDF·m^3·kg^{-1} can be found in some pixels in the Middle and Lower Yangtze River, Southern Deccan Plateau, and Amazonas High Andes, compared to their marginal EF close to 0. The average EF of some regions in Lena was lower than 100 PDF·m^3·kg^{-1} due to little increase in N concentration during the past 110 years.

Figure 4.2 Marginal effect factors (EFs) at a 0.5×0.5° resolution. The boundaries of the background map represent countries. Note that the right limit is contained, while the

left limit is not contained in the segment; e.g., 0 – 100 means 0< EF ≤100.
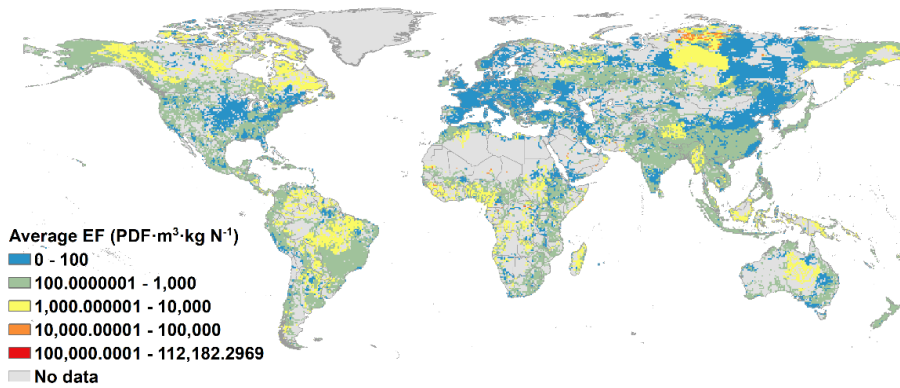
Figure 4.3 Average effect factors (EFs) at a 0.5×0.5° resolution. The boundaries of the background map represent countries. Note that the right limit is contained, while the left limit is not contained in the segment; e.g., 0 – 100 means 0< EF ≤100.

4.4 Discussion

This study is the first to showcase a regionalized (ecoregion-level) relationship between freshwater species loss and N concentrations. Such information can provide more local support for assessing eutrophication and ecotoxicity impacts on local biodiversity.

Our study also provides the EFs at a much finer resolution (0.5×0.5 degree) than common in previous studies, such as Cosme and Hauschild (2017), LC-IMPACT (Azevedo et al., 2020), and ReCiPe2016 (Huijbregts et al., 2017). It allows for providing more detailed information on the local ecosystems and supports future research on assessing the impacts of nutrient emissions on biodiversity, for instance, when integrated into life cycle impact assessments.

Since no study established EFs or sensitivity to N inputs for freshwater species, we compared the spatial variability of our results with EFs for marine eutrophication (Cosme and Hauschild, 2017) and freshwater fish sensitivity to P (Azevedo et al., 2013).

In all these studies, N and P emissions tie to densely populated regions and induce eutrophication (and toxicity) downstream in both freshwater and marine ecosystems. In line with marine EFs for N estimated by Cosme and Hauschild, (2017), our EFs for N for freshwater fish are higher in tropical regions than in temperate zones. Our results also agreed with their patterns of EFs increasing from the polar to the tropical regions for most regions of Eurasia, Africa, and South America; differently, higher EFs of our study in the polar region than in the temperate zone are observed for North America and North Asia. However, in these polar regions (e.g., Western Hudson Bay, Lena, and Taimyr), our results agree with Azevedo et al. (2013), who posit that heterotrophic species are more sensitive in cold regions since these species are adapted to low nutrient concentrations. These differences among studies may result from more uncertainty in SSDs for polar regions due to fewer species occurrence data there. These findings highlight the need to better assess the effects of high concentrations on species loss in the tropical zone, while at the same time, the vulnerability of species in cold regions should also be considered.

Our result on the PDF of the current state reflects the environmental threshold of N content for losing freshwater fish species. It shows a similar spatial pattern to the regional boundaries for N surplus (Schulte-Uebbing et al., 2022), which were also derived from IMAGE-GNM. A consensus on the most severe N exceedances exists in India/Pakistan, eastern China, the Nile Basin, areas in Saudi Arabia, and along the Peruvian coast. From our results and the comparison with other studies, it follows that using a finer scale allows for describing the nutrient effects on species in more detail but also influences the reflection of the realistic species sensitivity. Using broader regions erases the geographical distinction of natural communities' responses to the various environments in smaller regions and therefore overestimates the effect for hyposensitive ecosystems and underestimates the effect for hypersensitive ecosystems. Thus, we recommend calculating the SSDs for ecoregions, unless the ecoregions cannot derive SSDs due to a lack of data and have to apply the realm-and-major-habitat-level SSDs.

An underlying assumption of this study is that fish species loss is tied to N increase. However, the individual N limitation may also be affected by the co-limiting effects of N and P under influence of eutrophication (Du et al., 2020; McDowell et al., 2020). N effects may be overestimated by our method, since the disappearance of fish species in some areas is co-affected by other stressors such as P. As McDowell et al. (2020) found, 66% of the freshwater is limited by N. Even though the loss of species induced by P limitations or other stressors might have influenced the species occurrence at a certain N concentration at some locations, the species might still be observed at the same N concentration at other locations within the same ecoregion and would, therefore, be considered tolerant. This approach undermines the effect of co-stressors. For future studies, we suggest evaluating the effects on fish species richness by simultaneously considering other human pressures and especially the co-limitation effects due to P emissions. Observations may, e.g., be coupled to models of global P fate (Beusen et al., 2015), land-use change interactions (Comte et al., 2021), global warming impact (Barbarossa et al., 2021; Comte et al., 2021), and water consumption threats to freshwater fish communities (Pierrat et al., 2022). Such a coupling will help to further disentangle the direct impacts of nitrogen load and will help in understanding whether and how its effects interact with other biodiversity threats.

Furthermore, the species-sensitivity relations could be refined by increasing the number of species observations and the quality of global N predictions. Another source of uncertainty lies in potential sampling bias for our underlying point occurrence dataset. For instance, the present study encountered the same problem of lacking occurrence data as the previous studies in cold regions, where the species sensitivity could be overestimated due to the underestimation of species richness. The accessibility to more species occurrence records can lower the uncertainty in some ecoregions due to lack of data, e.g., Lena and Taimyr in Polar freshwater systems. The accuracy of IMAGE-GNM is tied to the uncertainty introduced in N concentration predictions and it can be affected by various reasons such as model inputs, retention models, and hydrological parameters. The predictions of N concentration can be upgraded by using a mechanistic model, such

as IMAGE-DGNM (Vilmin et al., 2020). The current version of IMAGE-DGNM has been applied for several watersheds, and a future version of global N modeling can better support the research on N-induced impact on fish biodiversity (Vilmin et al., 2020).

Last but not least, we used 1900 as the reference year, as it is the earliest simulated year in IMAGE-GNM, but ideally, the reference state could reflect the pre-industrial levels.

In conclusion, our study quantified the regional relationships between N enrichment in freshwater and fish species loss, which complements the current freshwater eutrophication studies based on P in LCA. The regionalized freshwater SSDs and EFs reveal the sensitivities of ecosystems to nutrient emissions at a fine resolution. They can be applied to assess the spatially differentiated biodiversity impacts of N emissions over the world in LCA.

Supporting Information

Ecoregion

Figure S4.1 Freshwater ecoregions

Fish occurrence (-)
1 - 10
10 - 100
100 - 1,000
1,000 - 10,000
10,000 - 383,456
No data
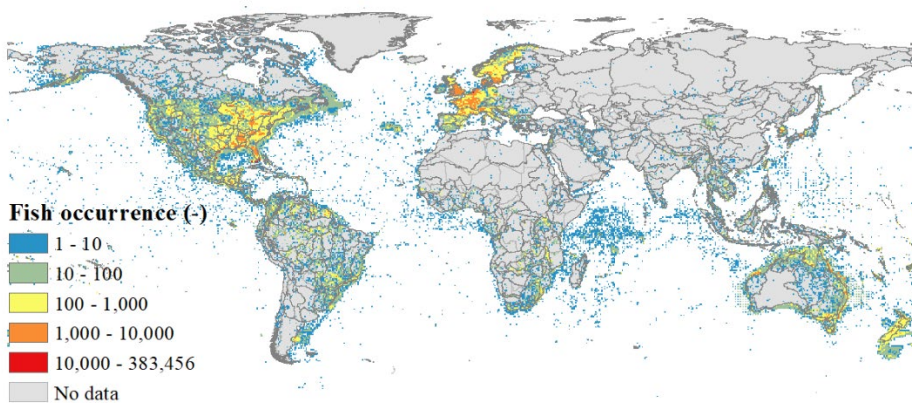
Figure S4.2 Occurrence records of freshwater fish species at a resolution of 0.5°×0.5°. The polygons illustrate freshwater ecoregions. We collected the occurrence records of freshwater fish species from 1970 to 2010, counted the numbers of the records during these 41 years, and rasterized them to a 0.5°×0.5° resolution. Fishes defined as freshwater species can be observed in freshwater, coastal, and marine ecosystems during their different life stages. We excluded the occurrences recorded in coastal and marine ecosystems when we matched the occurrence records with N concentration data in the freshwater.
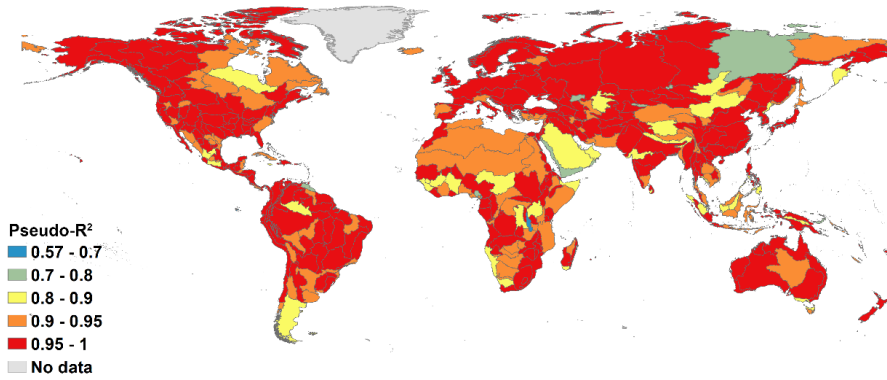
Figure S4.3 Cox-Snell pseudo-R^2 of species sensitivity distributions (SSDs) at the ecoregion level
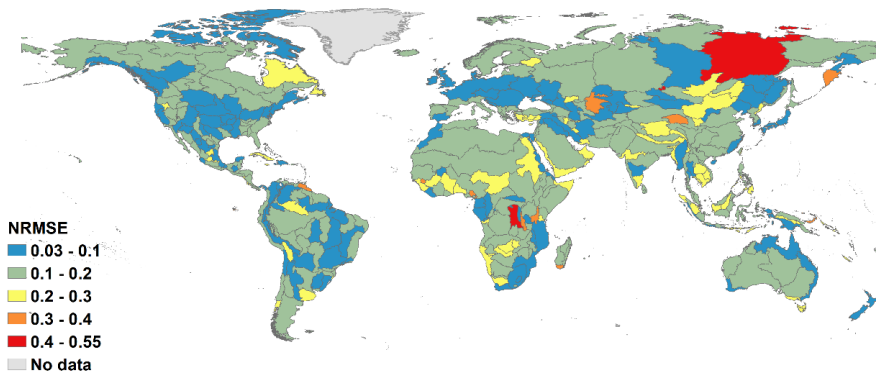
Figure S4.4 Normalized root mean square error (NRMSE) of species sensitivity distributions (SSDs) at the ecoregion level
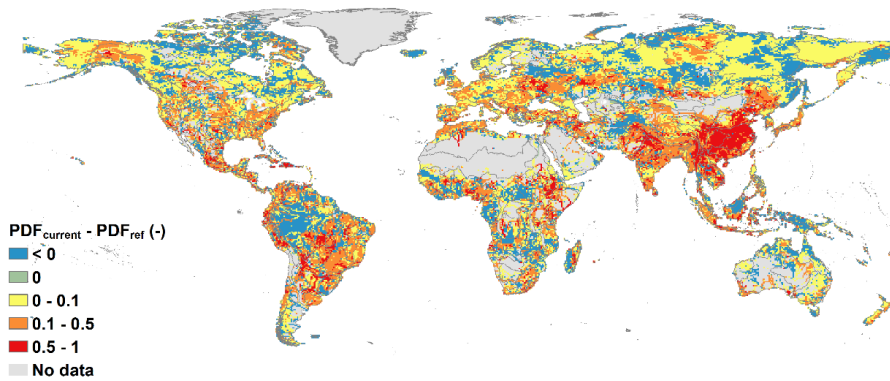
Figure S4.5 Change in the potentially disappeared fraction of species (PDF) between the current and reference states. In some grid cells, PDF decreases from the reference year 1900 to the current year 2010 along with a decrease of N concentration. This results from changes in hydrological conditions (e.g., change in discharge and/or runoff, land use change (e.g., conversion from agricultural land to natural land), and the inconsistency of the dataset whether there is ice cover or not (e.g., Iceland). In such cases, we set the average EF for these regions to "no value"
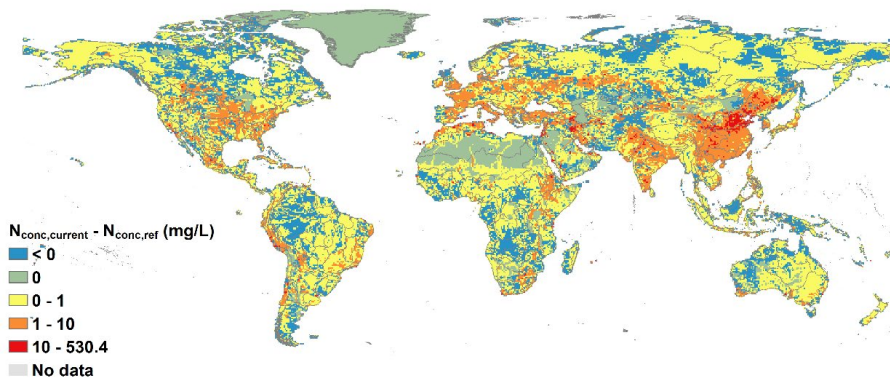
Figure S4.6 Change in N concentrations between the current and reference states